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(54) Title: FOOD PRODUCT AND PROCESS

(57) Abstract

A milk or other dairy product, capable of minimising the onset of disease such as coronary heart disease or enhancing the immune response is derived from animals which are substantially free of the  $\beta$ -casein A<sup>1</sup> allele. Bulk milk can be produced by testing for and culling cows who test positive for the  $\beta$ -casein A<sup>1</sup> allele, or by producing immunoglobulins and other immune response proteins, in cow's milk from animals not possessing the  $\beta$ -casein A<sup>1</sup> allele, or other commercial milk producing animals, to this allele, to counteract the immunosuppressant substances present that are produced from it, in commercial milking cows such as Holsteins, together with its blending with non-treated milk or the recovery of such immunoproteins.

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## Food Product and Process

### FIELD OF THE INVENTION

This invention relates to the removal, or the production of immunoglobulins against, immunosuppressant substances present, or, produced from the milk of animals of the genus  
5 Bos, and more particularly the domestic dairy species of the group Bos taurus and their crosses with the group Bos Indicus, which are used for milk production, and which contain specific casein alleles.

### BACKGROUND OF THE INVENTION

#### Description of the Background Art

10 It has long been understood that the early lactation mammary secretions of certain species, known as colostrum, contains substances that prevent disease, whilst the immune system of the young of the species is developing. This is particularly true of the ruminants, such as the Bos family. However the ingestion of colostrum is not essential in the human. These substances were identified as proteins (globulins) with immuno-properties which became known as  
15 immunoglobulins, (B L Larsen Immunoglobulins of Mammary Secretions in Advanced Dairy Chemistry Volume 1 Proteins. Ed. PF Fox Elsevier ,1992).

Immunoglobulins are present in the serum and mammary secretions of all mammalian species as part of the immune defence system of the animal. The immunoglobulins are also known as antibodies and are produced by the body's immune system in response to the presence of  
20 substances called antigens, including a wide range of molecules, bacteria, viruses, cells and particles that do not express specific markers of 'self' called histocompatibility antigens. Molecular antigens are largely peptides, proteins and carbohydrates. The classic immune response involves the production of antibodies capable of neutralising these antigens.

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The term antigen is now widely used to indicate any molecule that can be specifically recognised by the adaptive elements of the immune system, that is by both B cells, which produce immunoglobulins and T cells which release substances such as cytokines, (Immunology, 3rd Edition, Ed. I Roitt, J Brostoff, D Male, Mosby, London, 1993).

- 5 There are five classes, or isotypes, of immunoglobulins all of which have a similar basic structure, but have differences in their organisational structure as well as the amino acid sequences present and carbohydrate groups present. In addition to the immunoglobulins there are present related immune system proteins. These are known as complement and they are a complex group of proteins which assist the function of antibodies. Their properties are described in the above texts. There are at least 11 proteins in the complement group some of which are expected to be present in milk at the milligram per 100 millilitre level.

There are numerous patents that have been filed which seek to:

1. isolate the immunoglobulins present in mammary secretions, particularly colostrum but also including milk and products derived from milk such as whey. Generally the species involved is the domestic cow, *Bos taurus*, but it may include sheep or goats.
2. produce an "immune milk" or "health food" incorporating the immunoglobulin proteins, either as a result of stimulating the milk producer's immune system by the addition or injection of substances into the animal's body, either once or systematically, which result in an immune response, or by concentrating the small amounts of immunoglobulins that are naturally present in milk-derived products. In the former case the immunoglobulins may be specific responses to the injection of pathogenic bacteria into the milk-producing animals.

Examples of these patents include:

- Japanese patent (1988) JP 63-133941, Hori T, Nishimoto K, Kimura M, Yommazaki N, describes a process in which immunoglobulins are collected by ultrafiltration from whey, the by-product of cheese or casein manufacture. The immunoglobulin content of powder derived from this process was found to contain about ten times the immunoglobulin content of dried human milk.

- UK Patent Application (1987) GB 2 179 947 Monsan PFE, Thibault PA, Brossad C, Bruvier CSJ describes a process for the extraction of proteins, preferably lactoferrin or

immunoglobulins from whey comprising concentration of the whey using ultrafiltration with a polysulphone membrane (with MW cut-off 25,000-50,000) followed by diafiltration. The retentate is then subject to adsorption of the retained proteins by ion exchange treatment using a weak cationic carboxymethyl resin at pH 5-8.5 and preferably at 7-8; and elution at the same pH.

European Patent Application (1984) EP 0 102 831 A<sup>1</sup>, Linggood MA, Porter P, Powell JR describes the immunisation of host animals with a range of E. Coli implicated in human gastroenteric disease and the production of immunoglobulins, and a synthetic milk containing the immunoglobulins that are specific responses to the inoculation of the host.

10 UK Patent Application (1987) 8729031, to R C Bottomley claims the production of a whey protein concentrate rich in immunoglobulins by the use of ultra-filtration through a membrane having a cut-off of 500,000 daltons which retains the immunoglobulins, or by subjecting whey to the action of an anion exchange resin which does not remove immunoglobulins so causing an increase in their concentration in the effluent.

15 European patent application (1989) EP 0 336 694 Beck LR, describes a process for extracting an anti-inflammatory factor from cow or ewe milk, taken from animals that have been previously immunised by the administration of bacterial antigens. The anti-inflammatory factor is then extracted from whey that has been subjected to ion-exchange chromatography and molecular sieve chromatography.

20 US patent (1992) 5 106 618 Beck LR, Kotler DP describes the production of a 'hyperimmune' milk obtained by inoculating a milk-producing animal with a non-protozoan bacterial antigens, collecting the milk from the animal and the pasteurising and concentrating prior to use.

US patents (1989) 4 879 110 and (1993) 5 194 255 Beck LR, Stolle RJ, describe a method for inducing the production of a milk anti-hypertensive factor in an animal such as a cow by  
25 injecting bacterial antigens into the animal. The anti-hypertension factor is isolated by (1) removing from the milk molecules having a molecular weight greater than 10,000 daltons;(2) fractionating by ion-exchange chromatography the effluent to obtain a negatively charged fraction;(3) fractionating the negatively charged material eluted from the ion-exchange column using molecular sieve chromatography and isolating the hypertensive fraction from the latter  
30 step by isoelectric precipitation.

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US patent (1980) 4,216,236 Mueller HR, Legier CN, Secretin MC, Blonay CN claims the incorporation of soluble proteins obtained from whey using an ultrafiltration step with membranes having a molecular weight cut-off between 1000 and 500,000 incorporating immunoglobulins or to which immunoglobulin powder or concentrate has been added.

- 5 US patent (1984) 4 490 290 Ganni MM, May K, Porter P, describes the recovery of one or more milk immunoglobulins by passing the milk through a re-usable immunoadsorbent column comprising an insoluble carrier material to which is bound a low-affinity monoclonal antibody specific to the antibody(ies) but not specific to any other common constituent of milk. The bound immunoglobulin(s) are released by eluting the immunosorbent with 4 M  $MgCl_2$ .

10 **Problem**

- Notwithstanding all these patents and the claimed benefits of their products there is a considerable body of evidence that links milk particularly of the *Bos taurus*, the domestic cow with allergy problems with young children, asthma, chronic immune disorders such as diabetes mellitis, and atherosclerosis. Recent studies have also linked increased consumption of casein  
15 with the formation of hepatic tumours in rats, due it appears to a depressed NK cell cytotoxic activity, Bell RC, et al Nutr Cancr 22:151-162,(1994).

- To date it has not been possible to identify any particular fraction or molecule that is responsible for disorders such as atherosclerosis, although the consumption of animal fats and their associated saturated fatty acids have been claimed to either cause, or contribute to,  
20 coronary heart disease, hypertension and obesity as is set out in most medical texts on these subjects and the Surgeon-General's Report on Nutrition and Health, DHHS Publication No 88-50210 (1988).

**OBJECT**

- 25 It is an object of this invention to provide an improved food product and/or process or one which will at least provide the public with a useful choice.

**DEFINITIONS**

" $\beta$ -casein A<sup>1</sup> Allele" is a term used herein in reference to one of the variant forms of the  $\beta$ -casein gene. Expression of the A<sup>1</sup> allele results in the production of " $\beta$ -casein A<sup>1</sup>".

Where reference is made to the presence of the  $\beta$ -casein A<sup>1</sup> allele in an individual or population  
5 it encompasses both homozygous and heterozygous genotypes with respect to that allele. Similarly, where reference is made to the presence of  $\beta$ -casein A<sup>1</sup> it encompasses phenotypes resulting from either a homozygous or heterozygous state with respect to the  $\beta$ -casein A<sup>1</sup> allele.

The term "Immune milk" is used herein reference to milk obtained from an animal that has been immunised to selectively induce for formation of immunoglobulins and other immune  
10 proteins, directed against specific bacterial and/or viral pathogens or other foreign antigens that are known to cause diseases, in its milk, such milk being used to prevent disease, within the milk drinker, by fortifying the body's natural resistance against specific disease-causing antigens.

This invention is applicable to all products derived from cattle (live or dead) which products  
15 are substantially free of  $\beta$ -casein A<sup>1</sup>, or contains immune response proteins (including immunoglobulins) to  $\beta$ -casein A<sup>1</sup>. This includes meat (including offal) blood and blood products (such as black pudding), casein, gelatin, milk and other dairy products, as well as manufactured products containing some or all of the foregoing examples (including whiteners for beverages that include some milk solids).

20 The term "processed dairy product(s)" is used herein to refer to dairy products derived from a source of bulk milk (ie from milk from more than one animal) and includes, but is not limited to:

- (a) bulk milk used to make cheese whether or not the milk has been pasteurised or sterilised prior to cheese making,
- 25 (b) milk powder(s),
- (c) milk fats,
- (d) milk solids,
- (e) casein(s), caseinate(s), and casein hydrolysates,
- (f) pasteurised, sterilised, preserved milks including microfiltered milks, UHT milks,

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- (g) low fat milks,
- (h) modified or enhanced milks,
- (i) ice-cream or other frozen dairy based confections,
- (j) fermented milk products such as yoghurt or quark,
- 5 (k) cheeses including full fat, partial de-fatted and fat-free processed cheeses,
- (l) milk whey,
- (m) food products enriched through the addition of milk products such as soups,
- (n) milk from which allergenic molecules have been removed,
- (o) confections such as chocolate,
- 10 (p) carbonated milk products, including those with added phosphate and/or citrate,
- (q) infant formulations which may contain full, partially de-fatted or nonfat milk together with a number of additional supplements,
- (r) liquid or powdered drink mixtures,
- (s) butter, buttermilk, buttermilk powder.

15

#### STATEMENT OF INVENTION

In one aspect the invention provides a food product derived from animals which product is substantially free of the  $\beta$ -casein A<sup>1</sup> allele, or  $\beta$ -casein A<sup>1</sup> expressed therefrom.

Preferably the food product is meat or meat related, and more preferably is derived from an animal which is substantially free of the  $\beta$ -casein A<sup>1</sup> allele.

20

In a particularly preferred form of the invention the food product is milk or other dairy product which is substantially free of  $\beta$ -casein A<sup>1</sup>.

In another aspect the invention provides a meat or dairy food product which contains the  $\beta$ -casein A<sup>2</sup> allele in preference to the  $\beta$ -casein A<sup>1</sup> allele.

25 In another aspect the invention provides a milk or other dairy product capable of minimising the onset of coronary heart disease characterised in that the milk or other dairy product is substantially free of  $\beta$ -casein A<sup>1</sup>, or its proteolytic or heat produced products.



In a further aspect the invention provides a process for producing milk or other dairy products which does not contain  $\beta$ -casein A<sup>1</sup> by testing the individual cows in a herd for the presence of the  $\beta$ -casein A<sup>1</sup> allele, or the presence of  $\beta$ -casein A<sup>1</sup> in their milk, and selectively culling from the herd those cows that test positive for the presence of the  $\beta$ -casein A<sup>1</sup> allele, or  $\beta$ -casein A<sup>1</sup>,  
5 until the bulk milk produced by the herd is substantially free of  $\beta$ -casein A<sup>1</sup>.

In a related aspect the invention provides a process for producing milk or other dairy products which does not contain  $\beta$ -casein A<sup>1</sup> by testing the individual cows in that herd for the presence of the  $\beta$ -casein A<sup>1</sup> allele or  $\beta$ -casein A<sup>1</sup> in their milk and subsequently employing breeding programmes which select against individual cows testing positive for the presence of the  $\beta$ -  
10 casein A<sup>1</sup> allele or  $\beta$ -casein A<sup>1</sup> until the bulk milk produced by the herd is substantially free of  $\beta$ -casein A<sup>1</sup>.

In a further related aspect the invention provides a process for producing milk or other dairy products which does not contain  $\beta$ -casein A<sup>1</sup> by testing individual cows in a herd for the presence of the  $\beta$ -casein A<sup>1</sup> allele or  $\beta$ -casein A<sup>1</sup> in their milk and utilising genetic engineering  
15 procedures to remove the  $\beta$ -casein A<sup>1</sup> allele or inhibit expression of  $\beta$ -casein A<sup>1</sup> therefrom.

In another aspect the invention provides milk and other dairy products which are substantially free of  $\beta$ -casein A<sup>1</sup>.

Optionally the dairy product is casein which is substantially free of  $\beta$ -casein A<sup>1</sup>. This may be used as a food for animals or humans.

20 In another aspect the invention provides a process for producing immunoglobulins and other immune response proteins, in cow's milk from animals not possessing the  $\beta$ -casein A<sup>1</sup> allele, or other commercial milk producing animals, to this allele, to counteract the immunosuppressant substances present that are produced from it, in commercial milking cows such as Holsteins, together with its blending with non-treated milk or the recovery of such immunoproteins.

25 In another aspect the invention provides immunoglobulins and other immunoproteins produced as a result of inoculating commercial milk producing animals with  $\beta$ -casein A<sup>1</sup>, its proteolytic hydrolysis products, or fragments thereof produced by other means.

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In another aspect the invention provides blended milk obtained from mixing the product of the present invention with milk from animals possessing the  $\beta$ -casein A<sup>1</sup> allele as part of their genetic make-up either at the factory or by running a mixed herd of such animals.

5 In a related aspect the invention provides an immune milk (as herein described), such immune milk being substantially free of  $\beta$ -casein A<sup>1</sup> and/or produced from cattle or other commercial milking animals lacking the  $\beta$ -casein A<sup>1</sup> allele.

Preferably the immunoglobulins active against  $\beta$ -casein A<sup>1</sup> and its proteolytic products, are recovered by ultrafiltration, ion exchange chromatography or an immunoadsorbent column.

10 The milk containing immunoglobulins active against  $\beta$ -casein A<sup>1</sup> and its proteolytic products may be in the form of whole milk, whole-milk powder, skim milk, skim milk powder, milk whey, yoghurt, cheese, or any other dairy product, or processed dairy product.

In another aspect the invention provides immunoglobulins produced by treating a homozygous  $\beta$ -casein A<sup>2</sup>, B, or C, or heterozygous mixture of A<sup>2</sup>, B, and C, cow, with any inoculum to produce immunoglobulins in the milk from a cow that does not contain the immunosuppressant  
15  $\beta$ -casein A<sup>1</sup> allele,  $\beta$ -casein A<sup>1</sup>, proteolytic fragments thereof or fragments thereof produced by other means.

In a yet further aspect the invention provides a method of reducing the onset of disease in an individual or a population which derives some of its food intake from milk or other dairy products by reducing or substantially eliminating the presence of  $\beta$ -casein A<sup>1</sup> in the diet of that  
20 population. This method is applicable to animals or humans.

It is believed that the invention is applicable to reducing the onset of diseases such as:

coronary heart disease, cerebrovascular diseases, duodenal ulcer, peptic ulcer, respiratory diseases, such as bronchitis, lung cancer, asthma's and pneumonia, diabetes, polyarthritis, chronica, Psoriasis, renal disease, systemic lupus erythematosus, chronic disorders of the  
25 immune system, and any disease where there is a seasonal variation in incidence or death rate.

Preferably the invention provides a method of reducing the onset of coronary heart disease in a human population which derives some of its food intake from milk or other dairy products by reducing or substantially eliminating the presence of  $\beta$ -casein A<sup>1</sup> in the diet of that population.

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In a yet further aspect the invention provides a method of enhancing the immune response or decreasing the immune suppression of an individual or a population who or which derives some of his/her/its food intake from milk or other dairy products by reducing or substantially eliminating the presence of  $\beta$ -casein A<sup>1</sup> in the diet of that individual or that population.

- 5 The subject of this invention is the identification of the class of proteins responsible for a number of disorders such as coronary heart disease (and others as described above), their neutralisation in cow's milk and the production of an immunoglobulin capable of partially overcoming some of the deleterious effects they(or it) engender(s) on the human body. This invention is not limited to a specific disease as the molecules concerned appear to act as
- 10 immunosuppressants to the body's immune system and their removal can only enhance the general well-being of the individual while at the same time providing specific relief to individual's whose genetic make-up is such that contact with these proteins or protein will bring about a specific response such as atherosclerosis or other chronic disorders of the immune system.

15

#### **The discovery that is the basis of this Invention**

- It has been reported that certain groups of peoples are not subject to the diseases described above, notwithstanding the fact that they consume considerable quantities of milk proteins. These people include the Tibetans, rural Gambians, the Masai and Samburu people of Kenya.
- 20 The latter peoples are also found not to suffer from obesity, even in old age. The only major difference between the milk consumed by the above people is that it is derived from Zebu, Bos Indicus, and Yak, Bos Mutus. Neither milk contains the casein allele described as  $\beta$ -casein A<sup>1</sup>. In addition, people such as the Eskimo do not suffer from diseases such as CHD compared with their dairy product consuming Danish countrymen as is illustrated in Table 1:

**Table 1. Age-adjusted differences in morbidity from chronic diseases between Greenland Eskimos and Danes**

	Eskimos/Danes
Acute myocardial infarction	1/10
Stroke	2/1
Psoriasis	1/20
Diabetes	rare
Bronchial asthma	1/25
Malignant disorders	1/1
Thyrotoxicosis	rare
Multiple sclerosis	0
Polyarthritis chronica	low

Acta Med Scand 208: 401-406, (1980)

- 15 These and other aspects of this invention, which should be considered in all its novel aspects, will become apparent from the following description, which is given by way of example only with reference to the preferred embodiments, and makes reference also to the following graphs:

Figure 1 is a graph entitled "The effect of food component on Ischaemic Heart Disease during 1985 for males aged 30-69". This shows the death rate of all ages per 100,000 of population, for a range of countries, based on the consumption of  $\beta$ -casein.

Figure 2 is a graph showing the effect of dairy protein consumption on Ischaemic Heart Disease for males aged 30-69 for the year 1985.

Figure 3 is a graph showing the effect of saturated fat consumption on Ischaemic Heart Disease for males aged 30-69 for the year 1985.

- 25 Figure 4 is a graph showing the effect of red meat consumption on Ischaemic Heart Disease for males aged 30-69 for the year 1985.

Figure 1 shows a very strong correlation between the consumption of the food component, identified as  $\beta$ -casein A<sup>1</sup> (discussed in more detail below), and the death rate. Whereas the overall dairy protein consumption (Figure 2) does not provide such a strong correlation nor does the effect of saturated fat consumption (Figure 3), nor the consumption of red meat (Figure 4) come anywhere close to the very strong correlation with the inventor has identified in relation to the consumption of  $\beta$ -casein A<sup>1</sup>, both between countries and within countries. In the states of the form West Germany Ischaemic Heart Disease death rates are found to correlate

directly with the consumption of  $\beta$ -casein A<sup>1</sup> (Table 1A). In this instance the composition of the state dairy herd have remained virtually constant from the 1950's through to the 1980's.

**Table 1A: CHD nutritional risk factors, Federal Republic of Germany based on Schleswig-Holstein**

	Saturated Fat	Cholesterol	Alcohol	Carbohydrates	Energy	$\beta$ -A <sup>1</sup>	Rel IHD est.
Schleswig Holstein	1.00	1.00	1.00	1.00	1.00	1.0	1.0
Niedersachsen	0.97	0.96	1.00	0.98	0.99	0.92	0.88
Nordrhein Westfalen	0.99	1.02	0.99	1.00	1.02	0.97	1.00
Hessen	0.95	0.96	0.98	0.98	0.98	0.75	0.74
Rheinland-Pfalz	0.95	0.99	1.00	1.02	1.0	0.87	0.78
Saarland	0.94	0.93	0.98	1.01	0.98	0.90	0.88
Baden Wurttemberg	0.93	1.02	1.02	1.05	1.03	0.50	0.72
Bayern	0.96	0.99	1.22	1.06	1.02	0.50	0.74

5

## DETAILED DESCRIPTION

Caseins constitute the majority of the milk proteins. Dairy cattle exhibit genetic polymorphism in their proteins. The heterogeneity of the caseins is further complicated by the fact that they are the products of co-dominant allele autosomal genes. Some indication of their number, and the major product fragments into which they are split by proteolytic action of a variety of enzymes, is illustrated by the  $\beta$ -caseins in Table 2.

10

**Table 2. The  $\beta$ -casein family of proteins**

	Former nomen.	recommended nomen.	source of fragment
	$\beta$ -casein A <sup>1</sup>	$\beta$ -CN A <sup>1</sup> -5P	---
5	$\beta$ -casein A <sup>2</sup>	$\beta$ -CN A <sup>2</sup> -5P	---
	$\beta$ -casein A <sup>3</sup>	$\beta$ -CN A <sup>3</sup> -5P	---
	$\beta$ -casein B	$\beta$ -CN B-5P	---
	$\beta$ -casein C	$\beta$ -CN C-4P	---
	$\beta$ -casein D	$\beta$ -CN D-4P	---
10	$\beta$ -casein E	$\beta$ -CN E-5P	---
	$\gamma_1$ -casein A <sup>1</sup>	$\beta$ -CN A <sup>1</sup> -1P(f29-209)	$\beta$ -CN A <sup>1</sup> -5P
	$\gamma_1$ -casein A <sup>2</sup>	$\beta$ -CN A <sup>2</sup> -1P(f29-209)	$\beta$ -CN A <sup>2</sup> -5P
	$\gamma_1$ -casein A <sup>3</sup>	$\beta$ -CN A <sup>3</sup> -1P(f29-209)	$\beta$ -CN A <sup>3</sup> -5P
	$\gamma_1$ -casein B	$\beta$ -CN B-1P(f29-209)	$\beta$ -CN B-5P
15	$\gamma_2$ -casein A <sup>2</sup>	$\beta$ -CN A <sup>2</sup> (f106-209)	$\beta$ -CN A <sup>1</sup> -5P or $\beta$ -CN A <sup>2</sup> -5P
	$\gamma_2$ -casein A <sup>3</sup>	$\beta$ -CN A <sup>3</sup> (f106-209)	$\beta$ -CN A <sup>3</sup> -5P
	$\gamma_2$ -casein B	$\beta$ -CN B (f106-209)	$\beta$ -CN B-5P
	$\gamma_3$ -casein A	$\beta$ -CN A (f108-209)	$\beta$ -CN A <sup>1</sup> -5P, $\beta$ -CN A <sup>2</sup> -5P or $\beta$ -CN A <sup>3</sup> -5P
20	$\gamma_3$ -casein B	$\beta$ -CN B (f108-209)	$\beta$ -CN B

In addition there are a number of proteose peptone components.

W N Eigel Nomenclature of Proteins of Cow's Milk: Fifth Revision

J. Dairy Science 67:1599-1631, (1984)

- 25 Most animals are heterozygous. That is their protein composition contains a mixture of the various alleles inherited from the genes of their sire and dam. It appears that the original cow from which the current domesticated species developed contained only the  $\beta$ -casein A<sup>2</sup> allele.  $\beta$ -casein A<sup>1</sup> differs from A<sup>2</sup> in containing the following additional amino acids proline<sub>34</sub> and histadine<sub>6</sub>. The corresponding A<sup>1</sup> allele is a relatively recent modification. However some
- 30 animals are homozygous, that is their proteins are of one type only; in the case of  $\beta$ -caseins either A<sup>1</sup>, A<sup>2</sup>, A<sup>3</sup>, or B, C, D or E.

Bovine milk is an important source of proteins and other nutrients required by humans and the common domestic cattle species such as the Holstein have greater quantities of the A<sup>1</sup> allele than any other  $\beta$ -casein allele. Approximately 84 percent of the present American dairy herd is

35 estimated to carry this allele.

In the graph shown in Figure 1 the consumption of  $\beta$ -casein A<sup>1</sup> (and its derived proteolysis products) are plotted against the incidence of ischaemic heart disease based on FAO Food

Balance Sheets 1979-81 and WHO Trends in Mortality for Selected Causes of Death 1985-1989 and other reported CHD data.

In Table 3 the effect of heating milk to 63 °C for 20-30 minutes, known as Holder Pasteurisation, is set out together with the corresponding rate of CHD.

Population group	Holder intro. year	Angina pectoris(API) mort. p mill.				Cerebral embolism and thrombosis(CET)			
		AP 1	AP2	AP3	Δ%	CET 1	CET 2	CET 3	Δ%
U.K									
Edinburgh	1923	1925	67	92	37.3 <sup>a</sup>	1924	174	236	35.6
Glasgow	1924	1924	56	91	62.5 <sup>a</sup>	1924	77	101	31.2
Dundee	1924	1925	42	64	52.4 <sup>a</sup>	1925	162	188	16.0
Aberdeen	1926	1926	91	135	48.4 <sup>a</sup>	1927	121	227	87.6
Lanarkshire	1935	1937	188	375	99.5 <sup>b</sup>	1938	153	193	26.1
(excluding Glasgow)	1947	1948	685	1185	73.0	1948	298	518	73.8
	1952	1954	1185	1523	28.5	1954	518	680	31.3
County of Sutherland	1954	1954	963	1710	77.9	1954	610	823	34.9
County of Bute	1956	1956	1610	2848	76.9	1956	955	1398	46.4
London Admin. County	1925	1925	31	112	<u>261.3<sup>c</sup></u>	1926	90	120	<u>33.3</u>
	Average increase				81.8				41.6
Norway									
Oslo	1922	1922	3	43	1333.3 <sup>d</sup>	not available			

Columns AP1 and CET1 denote the year of commencement of the sudden rise in the appropriate mortality.

Columns AP2 and CET2 denote the appropriate average mortality for the 4 years immediately preceding the year of introduction of pasteurisation.

Columns AP3 and CET3 denote the appropriate average mortality for the 4 years immediately succeeding the introduction of pasteurisation.

Δ% represents average increase.

<sup>a</sup> Possibly low because deaths ascribed to "coronary thrombosis" were not included in International List No. 89 in Scotland until 1931.

<sup>b</sup> Possibly enhanced as deaths ascribed to "coronary thrombosis" were now included in International List No. 94.

<sup>c</sup> Possibly enhanced because (i) after 1927 all deaths ascribed to "coronary thrombosis" were included-unlike those in Scotland-in International list No. 89 and (ii) the large London creameries introduced Holder pasteurisation during this period.

<sup>d</sup> Mortality ascribed to the following group of classifications: angina pectoris, infarctus cordis, sclerosis art. coron. cordis.

A proteolytic enzyme plasmin, which is naturally present in milk, and which is largely associated with the casein, is both increasingly active at higher temperatures and is quite heat stable. At 60 °C it has been demonstrated to have a relatively high rate of conversion of

caseins, preferentially  $\beta$ -caseins to a range of proteolysis products. The increased mortality rate, demonstrated in Table 3, as a result of heating of the milk is presumed to be due to the formation of further proteolysis products, in addition to those naturally present, during the heating phase.

5 It is possible however that the specific fragment of  $\beta$ -casein A<sup>1</sup> that is entering or effecting the body's immune system which result from an enzyme contained within a psychotropic bacterium, or spore forming bacterium, present in the milk. Both the ratio of  $\beta$ -casein A<sup>1</sup>/ $\beta$ -casein A<sup>2</sup> and the concentration of psychotropic bacteria vary seasonally in milk. This seasonal fluctuation is thought responsible for part of the seasonal fluctuation in the illnesses that we  
10 have noted above.

This work is further supported by the results of Bell Rc, Golemboski KA, Dietert RR, and Campbell TC, Nutrition and Cancer 22;(2),151-162,(1994) who found that when Fischer 344 rats were fed diets containing 6 percent and 22 percent casein after being injected with a liver cancer causing substance, aflatoxin, the percentage of animals developing liver cancer  
15 increased directly proportional to the increase in casein in the diet. They interpreted the results to suggest that a low protein diet might result in lower suppression of the natural killer cell cytotoxicity activity. With our knowledge we can re-interpret their data to suggest that based on our own observations on the effect of  $\beta$ -casein A<sup>1</sup> on immunosuppression in humans, its reduction in the rat's diet reduced cancer formation by a factor of four due to a dose specific  
20 effect on the rat's immune system.

The preferred forms of this invention comprises the elimination from milk of  $\beta$ -casein A<sup>1</sup> or its proteolysis products, or protein fragments formed in any other way, either 'in vitro' or 'in vivo' and which have immunosuppressant properties, by the use of immunoglobulins raised against  $\beta$ -casein A<sup>1</sup>, the removal of  $\beta$ -casein A<sup>1</sup> and the inactivation of plasmin and other proteolytic  
25 enzymes. The preferred forms of the invention represents a significant advance over existing treatments for atherosclerosis, and other generally chronic immunosuppressant diseases in that it will prevent their occurrence in the new-born who, when they are genetically susceptible, will in other circumstances develop the diseases as they age. In addition it is believe it will assist in the restoration of organs and cells in those people where the damage to the bodies'  
30 organs is not permanent, by removing the source of chronic immune suppression.



By the term treatment, for the purpose of this invention it is intended that the symptoms of the disorder be ameliorated or completely eliminated or, where genetic typing indicates that an individual is of high risk of developing a disorder, of ensuring that it does not develop.

## 5 Example 1

In its preferred form the treatment consists of inoculating a milk producing animal, that does not possess the  $\beta$ -casein A<sup>1</sup> allele, preferably one that is homozygous for the  $\beta$ -casein A<sup>2</sup> allele, preferably one that produces commercially feasible quantities of milk, such as a cow, sheep, goat, or zebu with  $\beta$ -casein A<sup>1</sup>, or its proteolysis products, or fragments thereof, produced in  
10 any other manner, so that antigens to the foreign  $\beta$ -casein A<sup>1</sup> protein are produced. These antigens may be produced either alone, or as part of a wider inoculation programme, to produce a milk with an enhanced antigen concentration as has been described in the Art. This antigen enhanced milk is then added to 'normal' milk, or milk, or milk products, whose  $\beta$ -casein A<sup>1</sup> content has been reduced, using techniques known to those skilled in the Art, to counteract the  
15 presence of the immune suppressing  $\beta$ -casein A<sup>1</sup> derived material. Alternatively the above antigen(s) may be recovered by one of the processes known to the Art and used as a food supplement in its own right either alone or as a food additive.

Because the immunoglobulins formed as a result of the inoculation programme are somewhat heat sensitive then care has to be exercised with the pasteurisation and handling of the final  
20 product if a powder is required as is described in the existing Art.

Alternatively, immunoglobulins and other antigens, are recovered from non  $\beta$ -casein A<sup>1</sup> containing milk by ultrafiltration, ion exchange chromatography either singly, or in combination, or by use of a suitable immunoabsorbent column, comprising an insoluble carrier material to which is bound a low-affinity monoclonal antibody specific to one or more milk  
25 immunoglobulins but not specific to any other common constituent of milk. Such milk may have been derived from an animal that has been inoculated with a vaccine derived from a bacteria such as E. coli, for example, or which has been inoculated with 'bacterial antigens', as described in the Art. Alternatively, enhanced quantities of antigens are produced as a result of the inoculation, or inoculation programme of  $\beta$ -casein A<sup>1</sup>-free animals, to provide a milk

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product with all the claims as described in the prior Art. This invention has the advantage over the existing Art that immunosuppressant proteins resulting from the presence of, or, derived from the  $\beta$ -casein A<sup>1</sup> allele are eliminated from the final milk, or milk-derived products.

Another alternative includes the use of a plasmin inhibitor, such as a protein like aprotonin, or other such inhibitors, known to the Art, which are added, either singly or in mixtures, to the milk, as part of the above invention, to suppress the formation of additional  $\beta$ -casein A<sup>1</sup> proteolysis products that would otherwise be formed during processing, and storage, prior to sale.

### Example 2

10 A milk or other dairy product according to the invention can be produced by testing individual cows in a dairy herd for the presence of the  $\beta$ -casein A<sup>1</sup> allele, or for the presence of  $\beta$ -casein A<sup>1</sup> in milk, and then selectively culling those cows returning a positive result, until the bulk milk produced by the herd is substantially free of  $\beta$ -casein A<sup>1</sup>. Alternatively, homozygous cattle containing the  $\beta$ -casein A<sup>2</sup> allele can be selectively bred so that the  $\beta$ -casein A<sup>1</sup> allele is  
15 eliminated from the herd.

An alternative approach to remove  $\beta$ -casein A<sup>1</sup> from bulk milk would involve separating cattle from existing herds which contain the  $\beta$ -casein A<sup>1</sup> allele, allowing the remainder of the herd (which are free of the  $\beta$ -casein A<sup>1</sup> allele) to be used for the production of bulk milk or other dairy products, and those cattle containing the A<sup>1</sup> allele to be used for the production of  
20 products for purposes other than human consumption. Such a segregation process within a herd may be facilitated by the use of ear tags or the like to mark individual animals.

### Industrial Application

The invention provides a useful food product capable of increasing the health of an individual, or the health of a population. In one aspect the invention provides a method of enhancing the  
25 immune response of an individual or a population who or which derives some of his/her/its food intake from milk or other dairy products, by reducing or substantially eliminating the presence of  $\beta$ -casein A<sup>1</sup> in the diet of that individual or that population.

In a particularly preferred form, the invention applies a method of reducing the onset of coronary heart disease in a human population which derives some of its food intake from milk or other dairy products by reducing or substantially eliminating the presence of  $\beta$ -casein A<sup>1</sup> within the diet of that population.

## 5    **ADVANTAGES**

By reducing or substantially eliminating the presence of  $\beta$ -casein A<sup>1</sup> in the diet of humans, it is believed that the immune response of an individual or a population may be enhanced, or immunosuppression reduced, increasing the general well-being of the individual or the population. It is believed that some individuals may be particularly susceptible to the presence  
10    of  $\beta$ -casein A<sup>1</sup>, and it may be possible to develop a test for such susceptible individuals, and to recommend that they reduce or eliminate their consumption of milk or other dairy products containing  $\beta$ -casein A<sup>1</sup>.

## **VARIATIONS**

Recognising that dairy products free of  $\beta$ -casein A<sup>1</sup> are desirable it is preferable to ensure that  
15    the animal from which the product is derived has been tested for the presence of the  $\beta$ -casein A<sup>1</sup> allele or  $\beta$  casein A<sup>1</sup> expressed therefrom and subsequent selective breeding programmes (selecting for  $\beta$ -casein A<sup>1</sup> negative animals) carried out to eliminate the presence of the  $\beta$ -casein A<sup>1</sup> from the herd. It will be recognised that such testing may be carried out in a number of ways without departing from the scope of the present invention.

20    Without departing from the scope of this invention an alternative approach to remove the  $\beta$ -casein A<sup>1</sup> allele from a herd may be carried out. Such an approach may include the screening of sperm to be used for the purpose of artificial insemination for the presence or absence of the  $\beta$ -casein A<sup>1</sup> allele and selecting against those sperm which contain this allele.

In addition to the methods of removing  $\beta$ -casein A<sup>1</sup> (or the  $\beta$ -casein A<sup>1</sup> allele), from meat  
25    products, milk and "processed dairy products" that have been disclosed herein it would be within the scope of this invention to use a number of alternative methods. Such alternative methods may involve the removal of  $\beta$ -casein A<sup>1</sup> from milk products via ultrafiltration

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techniques or by utilising a non-toxic chemical or enzymatic process to remove or inactivate  $\beta$ -casein A<sup>1</sup>.

Finally, it will be appreciated that various other alterations and modifications may be made to the foregoing without departing from the spirit or scope of this invention.

5

## CLAIMS:

1. A product derived from cattle which product is substantially free of the  $\beta$ -casein A<sup>1</sup> allele or  $\beta$ -casein A<sup>1</sup> expressed therefrom.
- 5 2. A product derived from cattle as claimed in claim 1 wherein the product is derived from a dead animal and the product or the animal has been tested to establish that the product is substantially free of  $\beta$ -casein A<sup>1</sup> or the animal from which the product has been derived is substantially free of the  $\beta$ -casein A<sup>1</sup> allele.
- 10 3. A product derived from cattle as claimed in claim 1 wherein the product is meat (or offal) or a blood product, derived from a slaughtered animal and the product or the animal has been tested to establish that the product is substantially free of  $\beta$ -casein A<sup>1</sup> or the animal from which the product has been derived is substantially free of the  $\beta$ -casein A<sup>1</sup> allele.
- 15 4. A product derived from cattle as claimed in claim 1 wherein said product is a "processed dairy product" (as herein defined) substantially free of  $\beta$ -casein A<sup>1</sup>.
5. An immune milk (as herein described), such immune milk being substantially free of  $\beta$ -casein A<sup>1</sup> and/or produced from cattle or other commercial milk producing animals lacking the  $\beta$ -casein A<sup>1</sup> allele.
- 20 6. The use of dairy products which are substantially free of  $\beta$ -casein A<sup>1</sup> in the preparation of a food or medicine.
7. The use of "processed dairy products" (as herein defined) which are substantially free of  $\beta$ -casein A<sup>1</sup> in the preparation of a food or medicine capable of
  - (a) enhancing the immune response, and/or
  - (b) minimising or reducing the onset of diseases such as:  
25 coronary heart disease, cerebrovascular diseases, duodenal ulcer, peptic ulcer, respiratory diseases, such as bronchitis, lung cancer, asthma's and pneumonia, diabetes, polyarthritis, chronica, Psoriasis, renal disease, systemic lupus erythematosus, and chronic disorders of the immune system, and the like.

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8. A process for producing dairy products (including milk) from a herd of cattle which dairy products is or are substantially free of  $\beta$ -casein A<sup>1</sup> by testing the individual cows in a herd for the presence of the  $\beta$ -casein A<sup>1</sup> allele, or the presence of  $\beta$ -casein A<sup>1</sup> in their milk, and selectively culling or breeding those cows showing a positive response to the presence of the  $\beta$ -casein A<sup>1</sup> allele, or  $\beta$ -casein A<sup>1</sup>, from the herd until the bulk milk produced by the herd is substantially free of  $\beta$ -casein A<sup>1</sup>, and producing the dairy products (including milk for sale) from the bulk milk.
9. A process for producing dairy products (including milk) as claimed in claim 8 wherein the  $\beta$ -casein A<sup>1</sup> allele has been removed or the expression of  $\beta$ -casein A<sup>1</sup> therefrom inhibited by employing selected genetic engineering techniques.
10. A product derived from commercial milk producing animals which product contains or consists of immune response proteins (including immunoglobulins) to  $\beta$ -casein A<sup>1</sup>, its proteolytic hydrolysis products or fragments thereof produced by other means.
11. A product derived from cattle as claimed in claim 1 wherein said product is a "processed dairy product" (as herein defined) substantially free of  $\beta$ -casein A<sup>1</sup>.
12. A bovine animal which after being tested for the presence of the  $\beta$ -casein A<sup>1</sup> allele or  $\beta$ -casein A<sup>1</sup> has been identified as being free of the  $\beta$ -casein A<sup>1</sup> allele or  $\beta$ -casein A<sup>1</sup> expressed therefrom.
13. A product derived from commercial milk producing animals as claimed in claim 10 comprising a blended milk product containing milk which may contain  $\beta$ -casein A<sup>1</sup> and milk containing immune response proteins (including immunoglobulins) to  $\beta$ -casein A<sup>1</sup>.
14. A process for producing immune response proteins (including immunoglobulins) to  $\beta$ -casein A<sup>1</sup>, in cow's milk from animals not possessing the  $\beta$ -casein A<sup>1</sup> allele, or other commercial milk producing animals not possessing the  $\beta$ -casein A<sup>1</sup> allele, by inoculating commercial milk producing animals with  $\beta$ -casein A<sup>1</sup>, its proteolytic hydrolysis products, or fragments thereof produced by other means.
15. A process for producing immune response proteins as claimed in claim 14, wherein immunoglobulins are produced by treating a homozygous  $\beta$ -casein A<sup>2</sup>, B, or C, or

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heterozygous mixture of A<sup>2</sup>, B, and C, cow (or herd of cows), with an inoculum to produce antibodies to  $\beta$ -casein A<sup>1</sup>, its proteolytic hydrolysis products or fragments thereof produced by other means.

16. A method of enhancing the health of or reducing the onset of disease in individual animals or individual humans or populations of animals or humans which individuals or populations derive some of their food intake from products derived from cattle including meat, milk or other dairy products, by reducing or substantially eliminating the presence of  $\beta$ -casein A<sup>1</sup> in the diet of that individual or that population.
17. A method of (a) enhancing the immune response of animals or humans, and/or (b) minimising or reducing the onset of diseases in animals or humans such as:
- coronary heart disease, obesity, hypertension, cerebrovascular diseases, stomach or gastric cancer, duodenal ulcer, peptic ulcer, respiratory diseases, such as bronchitis, lung cancer, asthma's and pneumonia, diabetes, polyarthritis, chronica, Psoriasis, renal disease, systemic lupus, erythematosus, and chronic disorders of the immune system, and the like, by
- (i) reducing or substantially eliminating the presence of  $\beta$ -casein A<sup>1</sup> in the diet of that individual or that population, or
  - (ii) supplying the individual or population with sufficient immune response proteins (including immunoglobulins) to  $\beta$ -casein A<sup>1</sup> to counteract the presence of the  $\beta$ -casein A<sup>1</sup> in their diet, or
  - (iii) inoculating the animal(s) or human(s) to produce immune response proteins (including immunoglobulins) to  $\beta$ -casein A<sup>1</sup> to counteract the presence of  $\beta$ -casein A<sup>1</sup> in their diet, or
  - (iv) supplying the individual or population with a blended milk product (or dairy product derived from such a blended milk product) containing milk which may contain  $\beta$ -casein A<sup>1</sup> and milk containing immune response proteins (including immunoglobulins) to  $\beta$ -casein A<sup>1</sup>.

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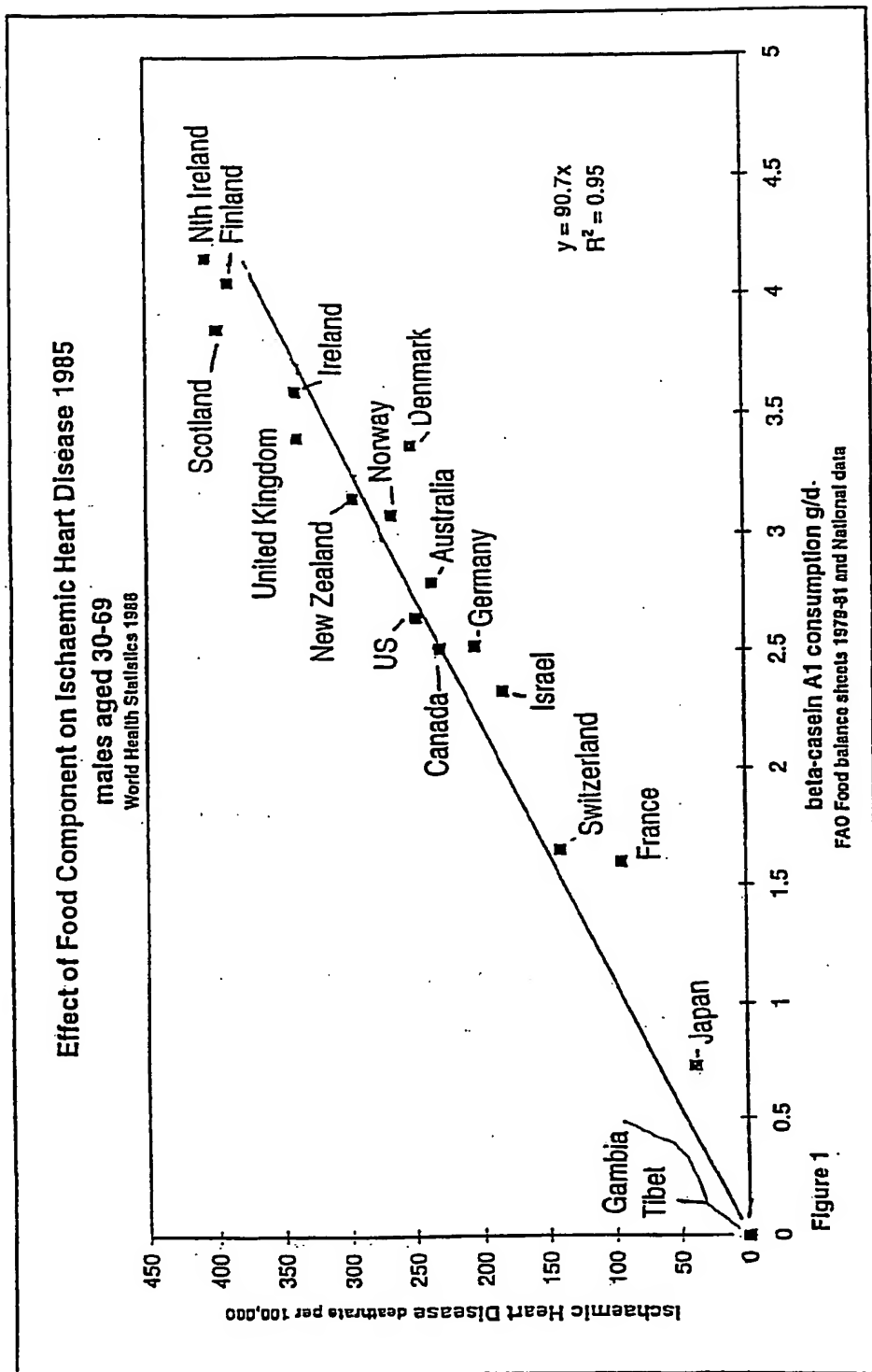


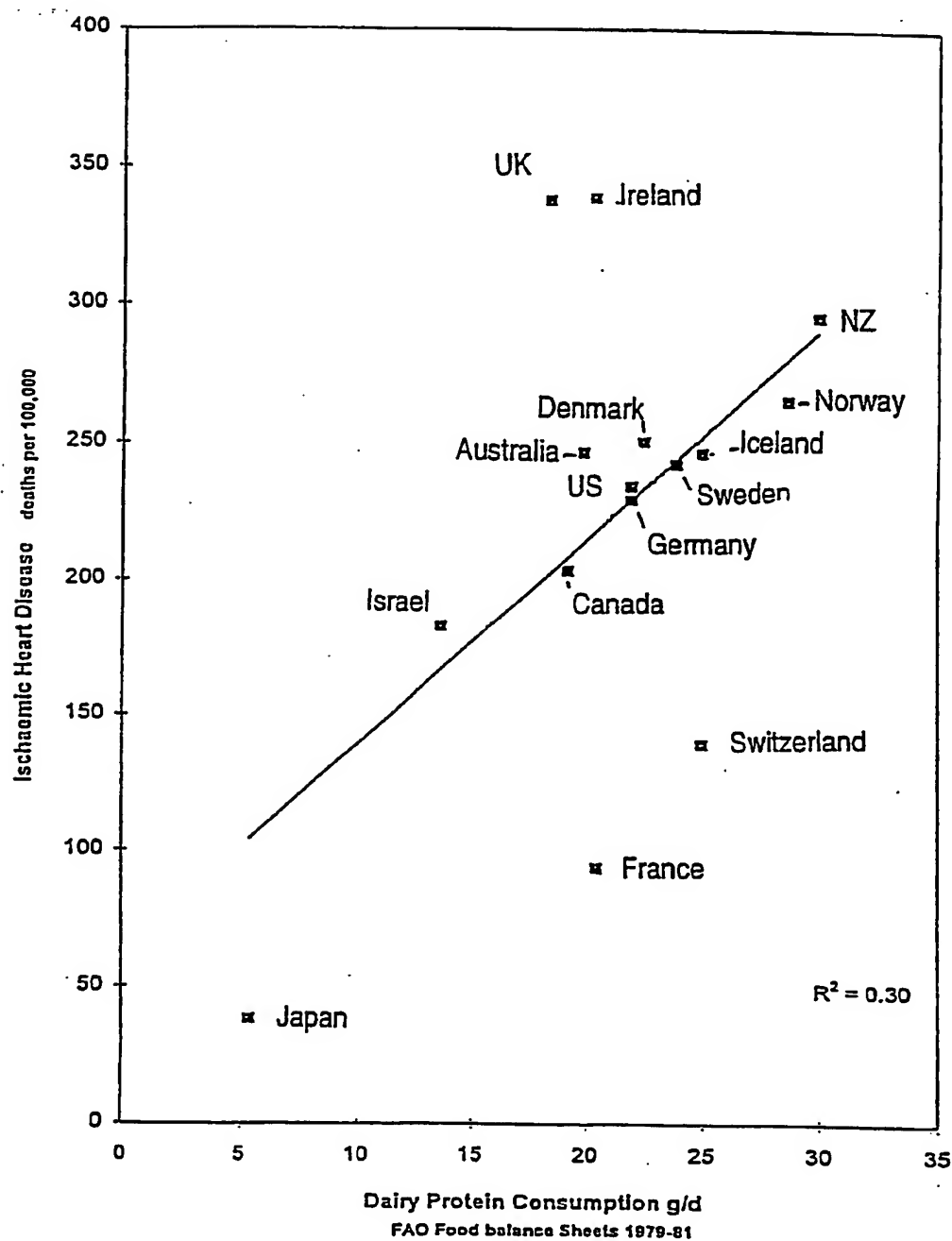
FIGURE 1



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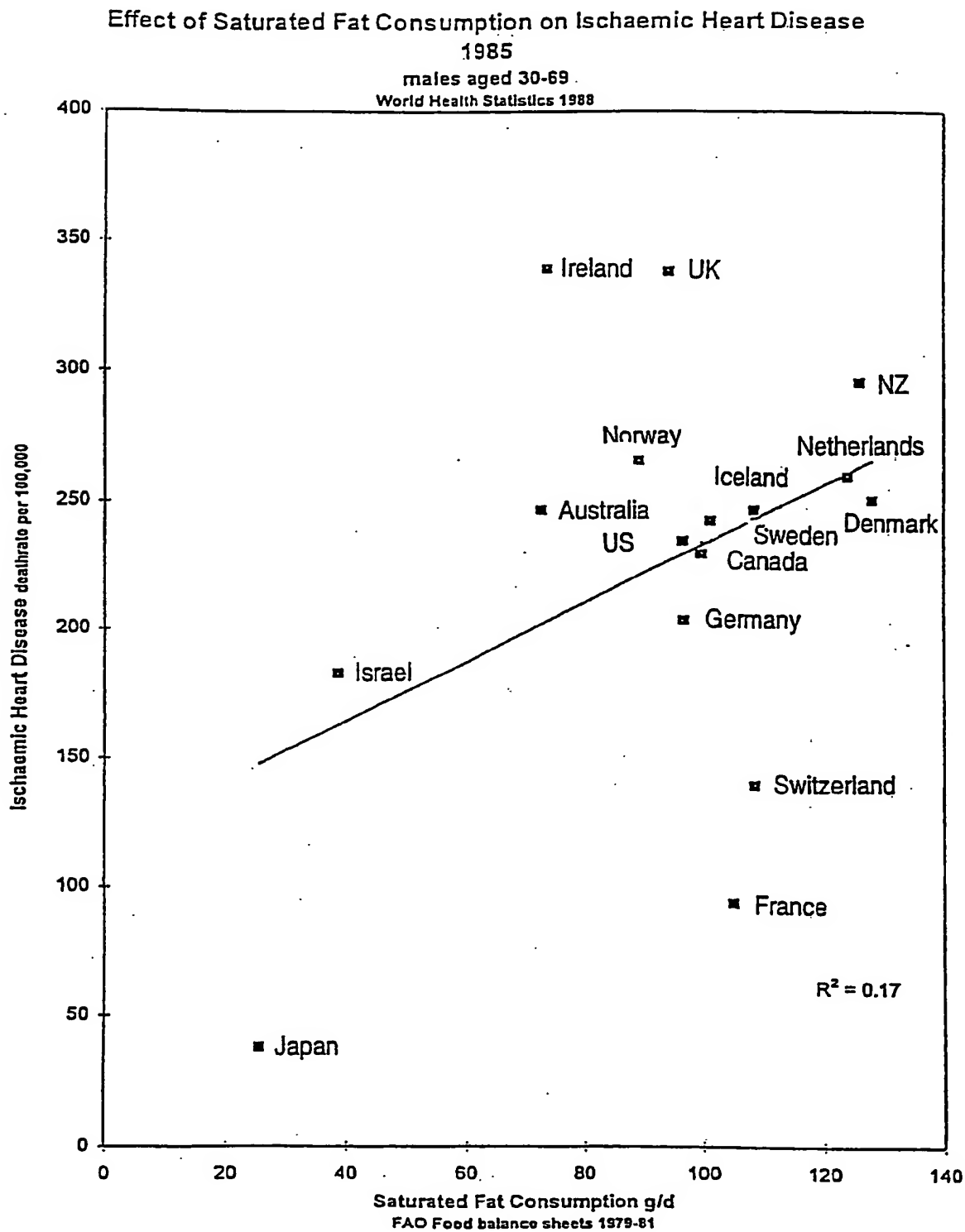
FIGURE 2

Effect of Dairy Protein Consumption on Ischaemic Heart Disease  
1985  
males 30-69 World Health Statistics 1988.



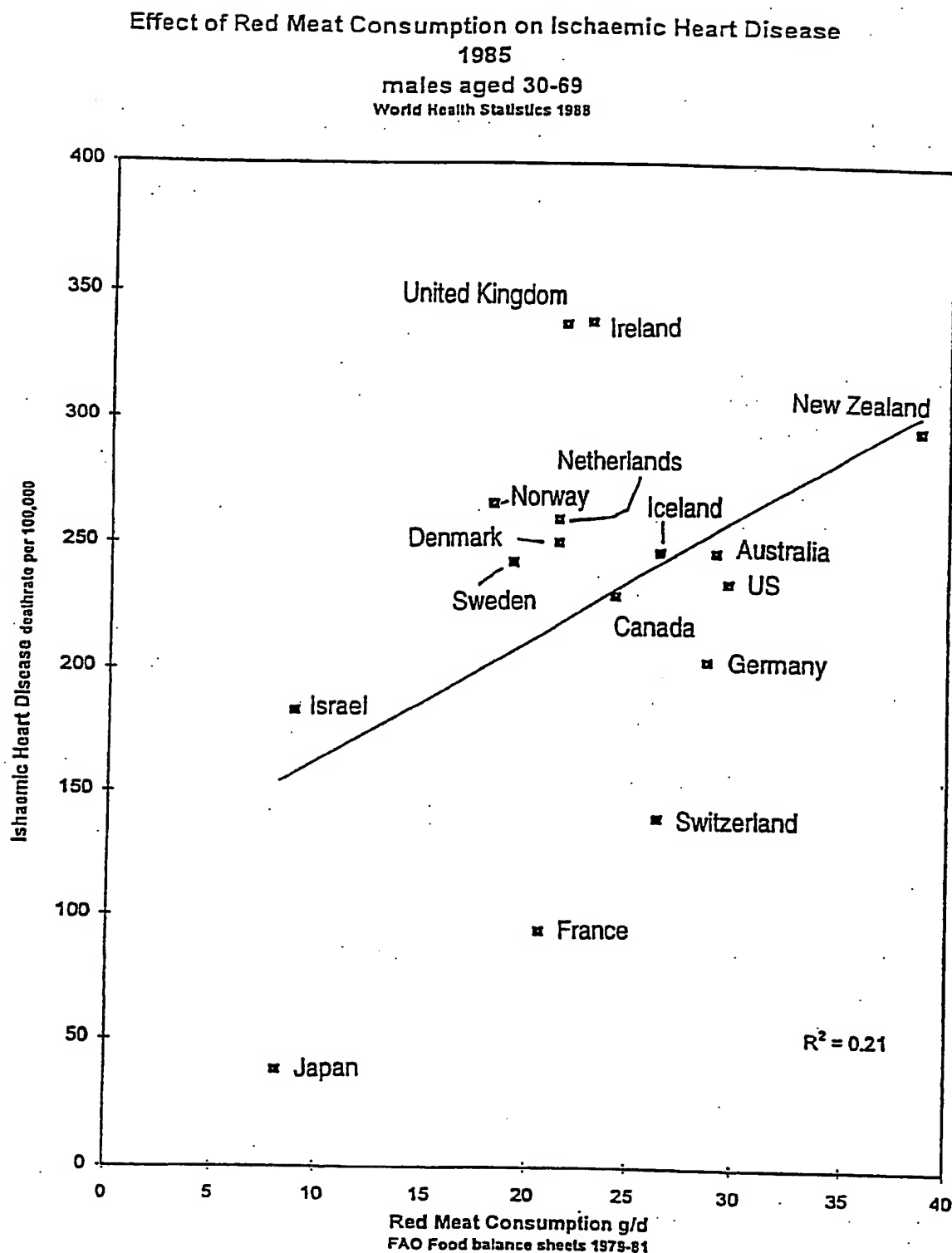
3/4

FIGURE 3



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FIGURE 4



# INTERNATIONAL SEARCH REPORT

International Application No.  
PCT/NZ 96/00039

<b>A. CLASSIFICATION OF SUBJECT MATTER</b>				
Int Cl <sup>6</sup> : A23C 9/00, A23L 1/31, G01N 33/04, 33/68, 33/487, A01K 67/02, C12Q 1/68				
According to International Patent Classification (IPC) or to both national classification and IPC				
<b>B. FIELDS SEARCHED</b>				
Minimum documentation searched (classification system followed by classification symbols) IPC : A23C 9/00, 9/15 A23L 1/31 G01N 33/04, 33/68, 33/48				
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched AU : IPC as above				
Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) DERWENT FSTA				
<b>C. DOCUMENTS CONSIDERED TO BE RELEVANT</b>				
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.		
X	AU,A, 76590/94 (VIABLE BIOPRODUCTS LTD) 20 April 1995 whole document	1, 4-7, 11, 16, 17		
E	WO,A, 96/14577 (THE NATIONAL CHILD HEALTH RESEARCH FOUNDATION et al) 17 May 1996 whole document	1, 4-8, 11, 12, 16, 17		
A	EP,A1, 631731 (BRISTOL-MYERS SQUIBB COMPANY) 4 January 1995 whole document			
<input type="checkbox"/> Further documents are listed in the continuation of Box C <span style="margin-left: 100px;"><input checked="" type="checkbox"/> See patent family annex</span>				
<table style="width: 100%; border: none;"> <tr> <td style="width: 50%; vertical-align: top;"> <p>* Special categories of cited documents:</p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> </td> <td style="width: 50%; vertical-align: top;"> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone</p> <p>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art</p> <p>"&amp;" document member of the same patent family</p> </td> </tr> </table>			<p>* Special categories of cited documents:</p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p>	<p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone</p> <p>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art</p> <p>"&amp;" document member of the same patent family</p>
<p>* Special categories of cited documents:</p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p>	<p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone</p> <p>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art</p> <p>"&amp;" document member of the same patent family</p>			
Date of the actual completion of the international search 14 August 1996		Date of mailing of the international search report <b>22 AUG 1996</b>		
Name and mailing address of the ISA/AU AUSTRALIAN INDUSTRIAL PROPERTY ORGANISATION PO BOX 200 WODEN ACT 2606 AUSTRALIA Facsimile No.: (06) 285 3929		Authorized officer  <b>MATTHEW FRANCIS</b> Telephone No.: (06) 283 2424		

Form PCT/ISA/210 (second sheet) (July 1992) cophon

# INTERNATIONAL SEARCH REPORT

International Application No.

PCT/NZ 96/00039

Box 1

Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 8 and 12

because they relate to subject matter not required to be searched by this Authority, namely:  
biological processes for production of animals and animal varieties respectively.

2. ☐ Claims Nos.:

because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:

because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a)

Box II

Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

Form PCT/ISA/210 (continuation of first sheet(1)) (July 1992) coplin

### Information on patent family members

**PCT/NZ 96/00039**

Patent Document Cited in Search Report				Patent Family Member			
AU	76590/94	EP	723400	FI	934494	WO	9510192
WO	96/14577	AU	39395/95				
EP	631731	AU JP	65988/94 7023717	CA US	2126639 5405637	CN	1105818